SUPPORT FOR THE AMENDMENTS

Claim 1 has been amended to incorporate the limitation of Claim 11. Accordingly, support for amended Claim 1 can be found in Claims 1 and 11, as previously presented.

Claims 12, 16, and 17 have been amended for clarity. Thus, support for amended Claims 12, 16, and 17 can be found in the same claims, as previously presented.

No new matter has been added. Claims 1-9, 12-38, 41-43, 72, 73, and 75-86 are active in this application.

REMARKS

At the outset, Applicants wish to thank Examiner Patterson for indicating that Claims 11, 12, 15-17, and 20 are only objected to as being dependent on a rejected base claim and would be allowable if rewritten in independent form. Applicants submit that, in view of the present amendments and remarks, all of the pending claims are now allowable.

The rejection of Claims 1-10, 13-14, 18, 19, 21-38, 41-43, 72, and 73 under 35 U.S.C. §103(a) in view of Ejima et al and the rejection of Claims 1-10, 13-14, 18, 19, 21-38, 41-43, 72, 73, 76-83, 85, and 86 under 35 U.S.C. §103(a) in view of Ejima et al in view of Yokoyama et al have been, in part, obviated by appropriate amendment and are, in part, respectfully traversed. First, in connection to Claims 1-10, 13-14, 19, 21-37, 76-78, and 85, as explained above, Claim 1 has been amended to incorporate the limitations of Claim 11. Applicants submit that Claim 1 and the claims dependent thereon (Claims 2-10, 12-37, 76-78, and 85) are patentable for the same reasons that Claim 11 was indicated as being allowable.

As for Claims 38, 41-43, 72, 73, 76-83, and 86, Applicants note that Ejima et al only describes the purification of human ***interleukin-6*** ("hIL-6") by solubilizing an inclusion body under denaturing conditions and then refolding hIL-6. Thus, this reference is completely

unconcerned with ***transglutaminase***. In contrast, the present claims are directed toward various methods for producing ***transglutaminase*** which exhibits enzymatic activity.

As explained on page 2 of the present specification, it is difficult to make predictions about the ability to refold a protein. Thus, the disclosure relating to interleukin is of little or no relevance to transglutaminase.

Moreover, as has been previously argued, Ejima et al discloses, "the native conformation of hIL-6 was obtained by rapidly removing the denaturant from the oxidized hIL-6 solution without any dilution." (see column 1 on page 302). Ejima et al further disclose that removal without dilution effectively suppressed aggregation during the refolding process of hIL-6. However, in the paragraph spanning columns 1 and 2 on page 302 of Ejima et al also discloses: "The pellet of inclusion bodies was solubilized in 6M GdnHCL... Solubilized hIL-6 (1.5 mL) was rapidly diluted 10-fold..."

Applicants wonder how in the face of such contradictory teachings one of skill in the art would expect *any* results based on Ejima et al. In fact, the skilled artisan would likely dismiss the disclosure of Ejima et al as having any reasonable applicability to hIL-6, much less an enzyme like transglutaminase.

Yokoyama et al discloses certain methods for producing transglutaminase. In particular, this reference discloses producing recombinant transglutaminase in a microorganism which over expresses a particular type of chaperon.

On page 2 of the Office Action, the position is taken that Yokoyama et al teaches the formation of transglutaminase existing in an intermediate state as recited in Claim 38. However, the whole point of this reference is to produce already-active transglutaminase without passing through any denatured state. Thus, the method of Yokoyama et al avoids and

does not involve a "step for forming an intermediate transglutaminase structure" as recited in Claim 38.

For these reasons, this reference cannot make obvious Claims 38 and the claims dependent thereon (Claims 41-43, 72, 73, 79-81, and 86).

Accordingly, the rejections should be withdrawn.

The rejection of Claims 38, 41, 42, 72, 73, 75, 79-84, and 86 under 35 U.S.C. §102(e) in view of Yokoyama et al and the rejection of Claims 38, 41-43, 72, 73, 75, 79-84, and 86 under 35 U.S.C. §103(a) in view of Yokoyama et al are respectfully traversed. As explained above, Yokoyama et al discloses certain methods for producing transglutaminase. In particular, this reference discloses producing recombinant transglutaminase in a microorganism which over expresses a particular type of chaperon.

Although on page 2 of the Office Action, the position is taken that Yokoyama et al teaches the formation of transglutaminase existing in an intermediate state as recited in Claim 38, as also explained above, the whole point of this reference is to produce already-active transglutaminase without passing through any denatured state. Thus, the method of Yokoyama et al avoids and does not involve a "step for forming an intermediate transglutaminase structure" as recited in Claim 38.

From the remarks on page 3 of the Office Action, it appears that it has been assumed that "an intermediate state" is produced by the method of Yokoyama et al. However, this assumption is incorrect. In order to produce an "intermediate state," a denatured transglutaminase needs to maintained in an acidic medium for 30 minutes or more. In the method of Yokoyama et al, the MTG is not kept in 8M urea at pH 5.5. In addition, an "intermediate state" does not coagulate and is recovered in the supernatant when it is dissolved in 8M urea buffer and then diluted. The recovery rate is not disclosed in

Yokoyama et al. However, it is assumed to be around 15%, because another published paper by the same authors ("Overproduction of Microbial Tranglutaminase in Eschericia coli, In Vitro Refolding, and Characterization of the Refolded Form," Biosci. Biothechnol. Biochem., vol. 64(6), pp. 1263-1270 (2000), which is based on Yokoyama et al and was submitted in the Information Disclosure Statement filed on May 23, 2002) discloses that it is about 15%. This recovery (around 15%) is low in comparison with those of the present examples (about 40 to 93%) and indicates that an "intermediate state" is not produced. Thus, the present claims are certainly novel over Yokoyama et al.

On page 3 of the Office Action, it is also asserted that 2 mg/ml is not far below 10 mg/ml. However, this difference in concentration is significant and meaningful in the light of commercial production. If the concentration is 5 times greater, then the production efficiency is significantly improved. For example, the size of the tank is reduced to one fifth, the amount of buffer is reduced to nearly one fifth, the time and the cost of concentrating the enzyme solution is reduced to nearly one fifth, and so on. In addition, in the method of Yokoyama et al, 10 mg/ml of a denatured transglutaminase cannot be refolded since the enzyme is coagulated when diluted in 0.5M urea after dissolved in 8M urea. Therefore, the present invention provides a significant and unexpected result in regard to commercial production and is unobvious in view of Yokoyama et al.

Furthermore, a skilled artisan knows that the recovery of a protein as well as an enzyme is important in developing a method for producing an enzyme, because the production yield is described as follows:

Yield = Enzyme Activity x Recovery of Protein.

As mentioned above, the recovery of protein according to the present invention is from double to six times that of Yokoyama et al and, thus, the production yield according to the

present invention must be significantly improved, since the enzyme activities are almost equivalent. Accordingly, the present invention provides an significant and unexpected improvement as compared to Yokoyama et al.

For these reasons, this reference can neither anticipate nor make obvious Claims 38 and the claims dependent thereon (Claims 41-43, 72, 73, 79-81, and 86).

As for Claim 75 and the claims dependent thereon (Claims 82-84), there is no teaching in Yokoyama et al which would suggest the particular transglutaminase claimed in Claim 75. Specifically, there is nothing in Yokoyama et al which would suggest that the obtained transglutaminase has a molecular ellipticity any different from the native transglutaminase. In sharp contrast, Claim 75 explicitly recites that the transglutaminase has "a molecular ellipticity which is 30 to 70% of that of the native state." There is no disclosure or suggestion of any such transglutaminase in Yokoyama et al.

Accordingly, the rejections should be withdrawn.

Applicants submit that the application is now in condition for allowance, and early notification of such action is earnestly solicited.

Respectfully submitted,

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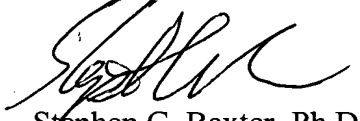

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